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Seasonal Variation of Fish and Macroinvertebrate Biomass Spectra in Southern West Virginia Streams

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

By

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Preface

This thesis was organized in a format suitable for publication in the peer-reviewed journal Freshwater Biology with minor modifications to comply with Virginia Commonwealth University guidelines for thesis submission.

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Abstract

SEASONAL VARIATION OF FISH AND MACROINVERTEBRATE BIOMASS SPECTRA IN SOUTHERN WEST VIRGINIA STREAMS

By: Andrew James Kirk, M.S. Biology

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The biomass size spectrum - the power-law scaling relationship between average individual size and total biomass - has often been studied in lake and marine ecosystems, but rarely in lotic systems. The objective of this study was to test for characteristic biomass spectra in small temperate streams. Seasonal fish and macroinvertebrate data, including population abundance and biomass estimates, were collected in three wadeable, southern West Virginia streams from October 2013 to May 2015. Fish abundances were estimated with 3-pass electrofishing (depletion) surveys and individuals were weighed in the field. Macroinvertebrates were collected with a Hess sampler and returned to the lab for identification to the lowest practical level (usually genus). Published length-mass regressions were then used to estimate individual mass. All size spectra relationships (linear regression of log-log data) were highly significant ($p<0.001$). Size spectra intercepts were variable and may reflect seasonal differences in fish and invertebrate densities. Size spectra slopes were more consistent, with a mean slope of approximately -0.73, suggesting a common scaling relationship between stream consumers at differing trophic levels.

Introduction

Organism abundances within ecological communities are often structured according to size, whereby the abundance of any species or cohort will tend to be inversely related to its average body size. This is largely due to predator-prey dynamics: predators consume prey that are typically smaller than themselves and multiple prey items are needed to sustain a single predator. Thus, relatively small organisms will necessarily be more abundant than larger organisms within a given system. Elton (1927) suggested these tenets could be usefully represented in four ways: food chains, size of food, niches, and the pyramid of numbers. Others, most notably Lindeman (1942), then built upon Elton's insights by characterizing the flow of energy through food chains when organized as discrete trophic levels.

In general, aquatic predators are gape-limited generalist consumers, ingesting food items that can be captured and handled with minimal effort, and are not restricted to any particular prey species (Werner & Gilliam, 1984; Kerr & Dickie, 2001). For example, Allan (1982) studied the diets of three stonefly species using gut content analyses within headwater streams and found that smaller stoneflies only consumed autotrophic material (algae) whereas larger stoneflies fed mainly on other invertebrates. However, Allan (1982) also noted that gut contents were variable for species among sites, suggesting that organisms are flexible in selecting the locally available resources they will consume, so long as those resources are compatible with physiologically determined size limits. Thus, assigning these stoneflies to a single species-level trophic level (i.e., primary or secondary consumers), without considering individual body size, would have

painted an incomplete picture of how different size-classes of stoneflies obtain their food. Similar size-based, ontogenetic shifts in feeding behavior have been documented for many aquatic organisms, particularly freshwater fishes (Werner & Gilliam, 1984; Schlosser, 1991).

These size-dependent shifts in feeding behaviors have led aquatic ecologists to emphasize the body size of the individual organism as a fundamental principal for ecological communities. Parsons (1969) proposed the use of a fundamental 'size spectrum' as a way to quantify the abundance or biomass of individuals within a common ecosystem, ultimately leading to a better understanding of the structure and community-level production of planktonic communities. The key innovation of this method was to treat all individual organisms within a given community or system as 'particles' of given size, thereby removing the effect of species' identity. Size spectra relationships are most often modeled as power-law functions of the form $Y = aM^b$, where Y is the variable of interest (e.g., metabolism, home range size, population biomass, or abundance), M is the average body mass of a single individual, b is a scaling coefficient, and a is a constant. For statistical convenience, the size spectrum is modeled using log-transformed Y and M data and linear equations of the form $\log(Y) = \log(a) + b \log(M)$, where b and $\log(a)$ become the linear model slope and intercept, respectively.

Kerr and Dickie (2001) presented a standard methodology for building and comparing size spectra models. This method focuses on co-occurring individuals within a single ecosystem and incorporates specimens from multiple trophic levels, seeking to model the distribution of biomass (B) or density (D) among trophic levels (Trebilco *et al*. 2013). Importantly, the method of Kerr and Dickie (2001) is 'ataxic': it uses individual-

level *M* data, irrespective of species identity, rather than species-level averages (White *et al*. 2007). And it utilizes the octave scale ($\log_2$ or doubling intervals) to partition individuals into size bins; the *D* of a given *M* interval is estimated by summing the number of individuals within that interval and *B* is calculated as the product of *M* (average *M* within the interval) and *D*. By ensuring that *M* scaling relationships from different systems are directly comparable, the method of Kerr and Dickie (2001) has enabled researchers to detect common patterns in the size-based distributions of *B* and *D* in a variety of marine and lentic ecosystems. For instance, Sprules and Munawar (1986) studied $B \propto M$ size spectra relationships for combined phytoplankton and zooplankton assemblages in the North Pacific central gyre, Lakes Superior, Huron, Ontario and Erie, and 25 inland Ontario lakes. They found that the slopes (*b* values) of the $B \propto M$ models were all statistically indistinguishable from -1.0 (when normalized *B* estimates and log-transformed *M* and *B* data were used; see below for information on the use of normalized *B* and *D* data). More recently, $B \propto M$ size spectra relationships have been used to study system-level effects of anthropogenic perturbations (Petchey & Belgrano 2010), such as size-selective harvest within marine fisheries (Jennings & Blanchard 2004).

In contrast to lentic systems, relatively little effort has been made to quantify and compare $B \propto M$ scaling relationships in streams (but see Poff *et al.*, 1993; Morin, 1997; Huryn & Benke, 2007). Here we present a seasonal (i.e., intra-annual) comparison of biomass size spectra within stream ecosystems to include macroinvertebrates and fishes. Stream biota are subjected to strong seasonal variations in environmental conditions (e.g., temperature and flow) and resource availability (Hemphill & Cooper, 1983; Meffe, 1984; Richardson 1991). For example, in many streams, major emergence events in late spring

and summer should significantly decrease invertebrate biomass through the autumn and winter months (Hynes, 1970). In contrast, many stream fishes spawn in the spring and summer months (e.g., Jenkins & Burkhead, 1994), and it is therefore logical to predict that the biomass of resident (non-migratory) fishes will increase through the fall months as juvenile fishes grow. Additionally, seasonal invertebrate drift and fish migration events related to spawning, wintering, and feeding may drive differences in the size-structure of seasonal communities (Brittain & Eikeland, 1988; Schlosser, 1991). For all of these reasons, it is plausible to predict that $B \propto M$ size spectra will exhibit seasonal or intra-annual variation.

Specific objectives of this study were to: (i) quantify macroinvertebrate and fish community structure in a historically understudied region; (ii) use the method of Kerr and Dickie (2001) to model $B \propto M$ size spectra in southern West Virginia Appalachian streams; (iii) compare size spectra models among seasons to determine whether seasonal changes in macroinvertebrate and/or fish B are reflected by consistent, measurable differences in the size spectra slopes and intercepts; and (iv) provide an empirical, seasonal baseline for comparison with other systems and to encourage other stream ecologists to consider the size spectra approach. Importantly, we were attempting to characterize 'natural' $B \propto M$ size spectra and therefore limited our study to minimally impacted streams.

Methods

Study area

Identifying minimally impacted sampling sites was a challenge because anthropogenic disturbance (e.g., mountaintop removal surface mining) is prevalent throughout the Appalchian Mountains in southern West Virginia (Bernhardt *et al.* 2012; Johnson, Fritz & Price, 2013). We therefore used the digital Critical Forest Map of Maxwell *et al*. (2012) to screen potential sampling sites. The Critical Forest Map is a grid-based representation of ecosystem health that uses multiple indicators of landscape structure, including land use/cover type, geomorphology, and degree of forest fragmentation, to calculate an integrated, categorical index of ecosystem integrity throughout the Southern Coal Fields region of West Virginia. Forest plots (i.e., grid cells) were ranked on an ordinal scale ranging from 0–3, with 3 being the least disturbed forest habitat. We were able to identify stream watersheds that were heavily populated by plots with Critical Forest scores of 2 or 3 by overlaying the Critical Forest Map on the 1:100,000 scale NHDPlus (Version 2) digital stream network (McKay et al. 2014) within a Geographic Information System (ArcMap 10.2). Three final sites were then selected from this subset, with the additional constraint that each site was located on public land to ensure fieldwork access (Figure 1). Additionally, we used the Stream Classification for the Appalachian region to classify our selected sites. (Olivero Sheldon, Barnett, & Anderson, 2015)

Cabin Creek (37.617° latitude, -81.454° longitude) is a 3rd order tributary of the Guyandotte River, located at the southern boundary of Twin Falls State Park (Wyoming County). In the surveyed reach, Cabin Creek consists of a steep series of pools, riffles, and runs with substrate dominated by large boulders and limited gravel distributed throughout the riffles and pools. An extensive hardwood canopy covers the entire study

reach. Camp Creek (37.550° latitude, -81.131° longitude) is a 4th order tributary of the Bluestone River that flows through Camp Creek State Park (Mercer County). Within the survey reach the stream channel is primarily riffles and runs with multiple shallow pools and one large, deep pool (>1.3 m). Canopy coverage is extensive and consists of temperate hardwoods. Substrate consists of medium-large boulders, flat cobbles in the riffles and runs, and some silt and sand deposits in the pools. Slaunch Fork (37.396° latitude, -81.889° longitude) is a 4th order tributary of the Tug Fork River, located near the West Virginia-Kentucky state line. The upstream watershed lies entirely within the state-protected Panther Wildlife Management Area (McDowell County) and is covered by dense stands of temperate hardwood. Within the survey reach, substrate is primarily a mix of cobble and gravel in riffles and runs, with sand and silt in two deep pools. County-level maps, photos, and summary information for all sites are provided in Appendix S1.

General survey design

Streams were surveyed at approximately quarterly intervals from October 2013 through May 2015, with the exception of early 2015 when flood conditions prevented sampling. At each site, a study reach of approximately 20× the mean wetted channel width, but no less than 100 m total length, was delineated. Channel width was measured perpendicular to the channel thalweg at 10–12 m intervals along the longitudinal profile. Channel gradient was also measured at the same longitudinal intervals using a stadia rod and Abney level. Water quality measurements, including pH, temperature, specific conductivity, and dissolved oxygen, were collected with a YSI Pro2030 (Yellow Springs,

Ohio) hand-held meter at the beginning of each sampling event. Complete stream channel and water quality data are provided in Appendix S1.

Fish and macroinvertebrate sampling

Fishes were sampled with a three-pass depletion survey design, using a Halltech HT-2000 battery backpack electrofisher (Guelph, Ontario). Closed survey reaches were established in each study stream by securing block nets at the lower and upper ends. During each pass, a 3-4 person crew moved upstream collecting as many stunned fishes as possible and transporting all captured fishes to a live well. Following each pass, the collected fishes were identified and measured for total body length (mm) and wet-weight (g). Fish wet-weights were subsequently converted to dry-mass estimates using the conversion factor of Waters (1977; 1 g wet-weight = 0.2 g dry-weight).

During each sampling event, six benthic macroinvertebrate samples were collected with a Hess sampler (500 μm mesh; 0.088 m^2 area). Individual Hess samples were distributed among a mix of riffle and run habitats. For each sample, the Hess was set to ~2-4 cm depth beneath the substrate and the internal substrate was agitated and scrubbed with a soft wire brush for 120 s. All Hess sample contents were preserved in 70% ethanol and returned to the lab for visual sorting in white trays with a 10× magnification lens. Individual specimens were then identified to genus or the lowest practical taxonomic level with a dissecting microscope. Dry mass estimates were inferred from individual head capsule widths, measured with an ocular micrometer. Published taxon-specific length-mass regressions (Smock 1980, Benke *et al*. 1999) were used in all dry mass calculations.

Data analyses

Following the standard method of Kerr and Dickie (2001), we used $\log_2$ size class bins to group similarly sized organisms by individual dry mass (*M*). Bins ranged from 6.4 $x10^{-3}$ mg to 2.14 x 10^5 mg for a total of 25 $\log_2$ *M* size classes. The smallest size class was set at 0.0064 mg because the Hess sampler was not efficient at collecting very small, meiofauna specimens below this size. Macroinvertebrates were pooled among the six Hess samples collected during each site × season sampling event. Individuals within the pooled samples were then partitioned by dry mass (following length-mass conversions; see above) among the $\log_2$ *M* bins. Notably, the size-based partitioning of macroinvertebrates was conducted in an entirely ataxic manner; each specimen was assigned to its corresponding $\log_2$ *M* bin without regard to its taxonomic identity. Summed macroinvertebrate abundances (*n*) within each of the $\log_2$ *M* bins were then converted to 1 m^2 density (*D*) estimates. Total biomass (*B*) within each $\log_2$ *M* bin was estimated as $D \times M$, where *M* was interpolated as the arithmetic mean of the upper and lower bounds of each $\log_2$ size interval.

Fish abundances (*n*) were estimated with the maximum likelihood Zippin (1958) method. Importantly, we did not calculate *n* for fish species. Rather, we calculated ataxic *n* estimates for 'populations' of individuals within each of the $\log_2$ *M* bins. In these ataxic calculations, which were logically consistent with the traditional 'individual particles' method of Kerr and Dickie (2001), individual fishes were identified only by their individual body mass. Thus, we used the Zippin (1958) method to estimate *n* within each of the $\log_2$ *M* bins that contained fishes; calculations included the number of specimens

captured in depletion runs 1, 2, and 3, and were performed independently for each of the $\log_2 M$ size classes (see Appendix S2 for a specific example). Fish n estimates for each of the $\log_2 M$ size classes were then converted to D estimates by dividing each n value by the stream channel surface area of the respective study site and standardizing the results to 1 m^2 values for direct comparison with the macroinvertebrate D estimates. As for macroinvertebrates, total B for each $\log_2 M$ bin was calculated as the product of $D \times M$. Note that size disparities between macroinvertebrates and fishes were large enough to preclude overlap within the same $\log_2 M$ bins; smaller bins (<30 mg M) were populated entirely by macroinvertebrates while larger bins (>50 mg M) were populated exclusively by fishes.

Prior to building $B \propto M$ size spectra models, all B and M values were 'normalized'. Normalization was necessary to account for the differing widths of the $\log_2 M$ bins; because the $\log_2 M$ bins become incrementally wider with increasing M (i.e., ΔM is not uniform among bins), traditional statistical procedures that are designed for continuous data, such as linear regression, will be biased (see Blanco *et al*. 1994; White *et al*. 2008). Normalization scales B to ΔM by dividing each B estimate by the width of its respective $\log_2 M$ bin (Vidondo *et al*. 1997; Kerr and Dickie 2001). This reveals the true shape of the $B \propto M$ distribution (see Fig. 2) and results in an unbiased estimate of the scaling exponent (b) from a power-law $B \propto M$ scaling model (White *et al*. 2008).

Ordinary least squares regression was then used to model normalized $B \propto M$ size spectra (NBSS) relationships following $\log_{10}$ transformation of all M and normalized B data. Instances of 'empty bins' (i.e., no individuals within a given $\log_2 M$ interval) were not included in size spectra regression analyses as their zero values unduly bias

regression model outputs (Blanco *et al*. 1994; White *et al*. 2008). For each site × season dataset (combined macroinvertebrate and fish data), we estimated the linear model slope (*b*) and intercept (log[*a*]). Finally, analysis of covariance (ANCOVA) was used to test for significant differences ($\alpha = 0.05$) among the slopes (*b*) and intercepts (*a*) of seasonal $B \propto M$ size spectra models. For each of the three sampling sites, a combined (all sampling dates) $B \propto M$ regression model was first built. Significant interactions between sampling dates and the slopes of the combined $B \propto M$ models were then taken as evidence of differing slopes among seasons (Crawley, 2007). If significant differences in the $B \propto M$ model slopes were not found, we used the same procedure to test for differences in the linear model intercepts. All linear regression and ANCOVA procedures were performed with R statistical software (R Core Team, 2014).

Results

All NBSS models were highly significant ($p \leq 0.001$), exhibiting a clear negative relationship between *M* and normalized *B*. An example of a typical NBSS model is shown in Figure 3 for the October 2013 macroinvertebrate and fish data from Slaunch Fork. NBSS model fits were also strong, with most $r^2 > 0.9$ (Table 1). Of the 18 site × season NBSS models, only one accounted for <85% of the variation in normalized *B* (Cabin Creek, August 2014; $r^2 = 0.68$; see Table 1). As expected, all of the NBSS model slope terms (*b* values) were negative and highly significant ($p \leq 0.001$). However, the significance of the intercept terms (*a* values) was more variable, with marginal levels in two of the NBSS models (Cabin Creek, October 2014 and Camp Creek, October 2014)

and no evidence of significance in two NBSS models (Cabin Creek, March 2014 and August 2014; see Table 1).

ANCOVA tests for differing NBSS model slopes were variable. The six NBSS models for Camp Creek revealed no evidence of differing slopes (slope x sample F = 0.029, p = 0.919); slopes for each of the seasonal NBSS models ranged from -0.85 to -0.76 (Table 1), with extensive overlap in the 95% confidence intervals (CIs; Figure 4). Marginal evidence for differing slopes (F = 2.293, p = 0.051) was detected in Slaunch Fork, where the NBSS slopes ranged from -0.80 to -0.62 (Table 1). NBSS slopes in Slaunch Fork were relatively shallow and marginally different than the March 2014 slope, as indicated by 95% CIs (Figure 4). Evidence for differing NBSS slopes was strongest in Cabin Creek (F =3.331, p = 0.008) where the model slopes ranged from -0.88 to -0.53 (Table 1). Particularly large differences between the May 2014 NBSS slope and the August 2014, October 2014, and May 2015 slopes were indicated by non-overlapping 95% CIs (Figure 4).

NBSS model intercepts were variable at all sites. Evidence for differences among the seasonal NBSS intercepts was strongest in Cabin Creek, where the October 2013 and May 2014 intercepts were substantially higher than the March 2014 intercept (Table 1). A formal ANCOVA significance test for the Cabin Creek intercepts was not appropriate, as the NBSS slopes were previously found to be significantly different. However, non-overlapping 95% CIs clearly indicated that the March 2014 intercept was not equivalent to the October 2013 and May 2014 intercepts (Figure 4). NBSS intercepts were relatively constant in Camp Creek, where most seasonal models bracketed an average intercept of ~0.8. The October 2014 NBSS intercept (0.34; Table 1) was, however, lower (F= 2.488,

$p = 0.036$) than the intercepts from the other Camp Creek models and significantly different than the May 2014 NBSS intercept (Figure 4). Finally, marginal evidence of differing NBSS model intercepts was found in Slaunch Fork. The lower bounds of the 95% CIs for October 2013, March 2014, and May 2014 intercepts exhibited little or no overlap with the 95% CIs for August 2014 and May 2015 intercepts, though the overall ANCOVA significance level was marginal ($F = 2.069$, $p = 0.075$; Figure 4).

Discussion

By characterizing seasonal NBSS relationships in three minimally impacted Appalachian streams, we have shown that the scaling of $B \propto M$ in small, forested lotic systems may be naturally variable among sites and seasons. Seasonal NBSS slopes were highly variable at one site (Cabin Creek), moderately variable at a second (Slaunch Fork), and relatively constant at a third (Camp Creek; see Figure 4). NBSS intercepts were also variable among sites and seasons, with the highest intra-annual variation observed at Cabin Creek (Figure 4).

The Camp Creek NBSS models provide the best argument against intra-annual variation of NBSS in lotic systems, suggesting that the distribution of biomass among body sizes is nearly constant as well as the total biomass in the system. The slope was highly consistent amongst seasons with confidence intervals greatly overlapping for all six sampling dates. NBSS model intercepts were similar as well with the noted exception of October 2014 being significantly lower than May 2014. While not significantly lower, the August 2014 intercept was less than May 2014 as well, but still greater than the

October 2014 intercept. This may indicate biomass is gradually reduced in the system from the spring until the fall in response to insect emergence patterns (Hynes, 1970).

Cabin Creek NBSS models were the most variable. The May 2014 NBSS slope (-0.876) was the steepest slope amongst the models, suggesting a greater proportion of the system's biomass is allocated amongst the smaller size classes (i.e. macroinvertebrates). When comparing size class densities of May 2014 to August 2014 – May 2015, greater invertebrate densities and lesser fish densities were collected in May 2014. Most notably we collected more invertebrate individuals in the 4.9 mg – 19.6 mg size classes in May 2014 than in any other Cabin Creek samples. These individuals could therefore have been approaching their emergence period. For example large (>4 mg) *Ephemera sp.* were prevalent in our May 2014 sample (data not shown) and members of mayfly family Ephemeridae exhibit synchronous and sporadic emergences (Brittain, 1982). Likewise, large dragonfly larvae (Gomphidae) were most prevalent in the May 2014 sample (data not shown). Gomphidae are considered to be semivoltine (less than one generation a year) and so it possible we collected late instar larvae in May 2014 that were ready to emerge (Corbet, Suhling & Soendgerath, 2006).

Slaunch Fork NBSS model intercepts and slopes were consistently higher and steeper, respectively, for October 2013 to May 2014 models when compared to the other three models suggesting greater overall biomass. Unlike Cabin Creek and Camp Creek data, we could not find any patterns in the size class differences amongst sampling dates beyond decreased abundances and biomass. However, flooding in March 2015 may explain differences between May 2014 and May 2015 NBSS models. It is possible that the scouring effect of this flood event removed a significant number or biomass of biota

from the reach and that these biota had not yet re-equilibrated prior to the May 2015 sampling event.

Interpreting among-site differences in the NBSS slopes and intercepts is also difficult because so little theory and/or empirical precedent currently exist for comparing NBSS in streams. Notably, a regional stream classification system that was recently completed for the entire Appalachian region identified two of our study streams (Cabin Creek and Camp Creek) as "medium gradient, transitionally cool with moderately variable hydrology." The third site (Slaunch Fork) was also classified as "medium gradient, transitionally cool", but with "high flow variability" (Olivero Sheldon, Barnett, & Anderson, 2015). In general, the similarity in the physical characteristics of each stream may create the expectation that NBSS slopes and intercepts would be similar among sites, but the variability of our NBSS results seem to contradict this.

NBSS comparisons and potential sources of bias

Established theory on the NBSS stems primarily from lake and marine ecosystems where the high abundance of small, planktonic organisms drove early interest in the use of an automated (Coulter counter), ataxic method to quantify community-level size structure (Boudreau *et al*., 1991; Sprules & Barth 2016). Comparison of our NBSS models with lake and marine examples should therefore provide a useful theoretical context for our results. The slopes of our 18 NBSS models were often variable among sites and seasons (Figure 4) but all of them were significantly lower (i.e., shallower) than the 'canonical' slope of approximately -1.0 reported by Sprules and Munawar (1986), Sprules and Goyke (1994), and Yurista *et al*. (2015) for lake and marine communities. If

the slopes of the NBSS models described here are truly unbiased estimates of the $B \propto M$ relationship in our study streams, they may indicate that the biomass of large organisms (i.e., fishes) increases more rapidly in Appalachian streams than in lake and marine environments (Emmrich *et al*. 2011).

However, it is also possible that the relatively shallow slopes of our NBSS models may reflect biased estimates of the biomass of some smaller organisms. For example, allochthonous resources such as terrestrial insects that fall into the water are often an important energetic subsidy for stream fishes (e.g., Baxter *et al.*, 2005; Utz & Hartman, 2007). If these subsidies constitute an important resource for fishes in our study streams, then our *B* estimates for several macroinvertebrate size classes will underestimate the true *B* values and result in shallower NBSS slopes (as the height of the Y-axis observations for some smaller size classes will be too low). Furthermore, our study did not include meiofauna, which can achieve very high biomass in some streams and strongly influence the overall slopes and intercepts of NBSS models (e.g., Poff *et al.*, 1993; Schmid, Tokeshi & Schmid-Arraya, 2002). If meiofaunal biomass was very high in the study streams, our NBSS model intercepts may be biased (driven by a failure to properly characterize *B* at the far-left side of the X-axis), which may in turn underestimate the steepness of the NBSS slopes (due to artificially low estimates of *B* at the smallest size classes).

One other potential source of bias in our NBSS models may be the length-mass conversion factors that were used to estimate individual macroinvertebrate dry mass from length measurements. We felt that the published, taxon-specific conversion factors (Smock 1980; Benke *et al*. 1999) used in this study were an optimal method because they

can account for differences in overall body morphology (e.g., taxa with long, thin abdomens versus taxa with short, stout bodies). Nevertheless, these conversions entailed an unknown amount of estimation error (relative to weighing individual specimens) and alternative methods are available. For example, building upon the particle counter and acoustic methods that have been used to estimate *B* for very small organisms in lake and marine systems (see Yurista *et al*., 2014), Morin and Nadon (1991) proposed an ataxic, volumetric conversion factor to estimate individual dry mass (*DM*) from body length (*L*): $DM = L^3$. This simple equation assumes that most invertebrates are approximately cylindrical in shape and it will necessarily underestimate the mass of specimens with more flattened bodies. But the *DM* equation of Morin and Nadon (1991) does highlight the importance of using standardized methods to build and compare NBSS models.

In general, the lack of a broadly recognized, standard method for conducting size spectra studies in lotic systems may be limiting the utility of the approach in stream ecology (Morin 1997). For instance, Schmid *et al*. (2000; 2002) studied the scaling of population density (*D*, which can be converted to *B*) with *M* in two European streams and reported strong, negative relationships with slopes close to -1. Their results are particularly notable because the sampling method used to quantify meiofauna and macroinvertebrate abundance was so comprehensive. Unfortunately, their *taxic* data (>250 taxa in each stream) cannot be directly compared with our ataxic, normalized $B \propto M$ scaling results. Huryn and Benke (2007) used ataxic macroinvertebrate data to compare $D \propto M$ scaling relationships in southeastern U.S. and New Zealand streams and reported significant negative slopes ranging from -0.59 to -0.99. However, they partitioned their ataxic data among $\log_5$, rather than $\log_2$, size classes and did not

normalize the resulting *D* estimates. Similarly, in a study of a 4th order Virginia (USA) stream, Poff *et al*. (1993) compiled ataxic meiofauna, macroinvertebrate, and fish data to model the $D \propto M$ relationship, but used un-normalized *D* estimates within $\log_{10}$ size classes.

Because stream size spectra studies have so often used different methods, direct comparisons with our NBSS data are difficult. Two previous investigations do, however, provide a useful comparative context. First, in a study of 12 streams in eastern Canada, Morin and Nadon (1991) used the same analytical method applied in our study (i.e., ataxic data partitioned among $\log_2$ size classes, normalized *B* estimates, and $B \propto M$ scaling relationships plotted on $\log_{10}$ axes) to examine the NBSS for combined periphyton and invertebrate samples (but exclusive of fish). They reported a modal or hump-shaped $B \propto M$ relationship, rather than a negative linear one. However, when they superimposed normalized *B* estimates for microscopic organisms from other stream studies (e.g., bacteria and ciliates), effectively extending the origin of the *x*-axis of their combined NBSS plot, they observed a clear linear relationship with an approximate slope of -1. Morin, Rodriguez, and Nadon (1995) then extended their initial results by collecting repeat samples from the same streams over a 15-month time period and found that the overall shape of the NBSS was nearly constant.

Second, despite methodological differences (un-normalized data within $\log_{10}$ size classes; see above), the study of Poff *et al*. (1993) can be compared with our own results by restructuring the data. When our raw fish and macroinvertebrate data are partitioned among $\log_{10}$, rather than $\log_2$, size classes and all of the *B* estimates are normalized (i.e., divided by the width of each $\log_{10}$ size interval), our data become more comparable to

the Poff *et al*. (1993) data. For example, Figure 5 shows NBSS relationships for Slaunch Fork, West Virginia (October 2013 data) and Goose Creek, Virginia (Poff *et al*., 1993) that were constructed with the same methodology. Notably, the standardized NBSS plot illustrates strong similarity in the $B \propto M$ scaling relationships between the two streams. This similarity must be interpreted with caution because the Goose Creek model was strongly influenced by meiofauna samples (black diamonds in Figure 5) and we did not include the meiofauna. Thus, we cannot say how much the Slaunch Fork NBSS might change with the addition of meiofauna data. Nevertheless, the clear similarities between our NBSS results and the data of Poff *et al*. (1993) and Morin and Nadon (1991) are intriguing and seem to suggest that *B* increases at approximately the same rate with *M* in multiple types of aquatic systems. Alternatively, if the moderately shallower or lower slopes of our NBSS models (approximately -0.8 to -0.6 vs. -1.0) are accurate, it may indicate that *B* increases at a slightly faster rate with *M* in streams than in lake or marine ecosystems.

Future NBSS research and applications

In addition to expanding the empirical database on NBSS, future research should attempt to explain changes in the NBSS parameters through space and time. For instance, Morin *et al.* (1995) conducted NBSS analyses on 12 streams over 15 months and averaged their NBSS of the streams for each sampling date to elucidate temporal variations. Most notably, they remarked that despite changes in species composition over the sampling period, the NBSS appeared stable and attributed the stability to the continuous recruitment of early instars during the spring through the fall seasons.

However, Watz *et al.* (2016) demonstrated ice cover affects the growth of brown trout (*Salmo trutta*). Through the establishment of plastic sheets over 30m stream length sections during the winter in central Sweden, they found that trout under ice cover exhibited substantially higher growth rates and greater daily movements due to decreased light intensities (which promoted more daytime foraging), reduced metabolic rate and reduced risk of predation from mammals and birds. If ice cover in streams promotes greater growth and survival for fish, it may be reasonable to hypothesize that higher elevation and/or latitude streams that ice over may exhibit greater fish biomass in the winter months than lowland streams.

Directly assessing the effects of terrestrial subsidies on NBSS in streams might prove particularly helpful in explaining seasonal changes in NBSS parameters. Detrital terrestrial subsidies from litter fall and/or runoff have been shown to significantly relate to invertebrate biomass and abundance (Wallace *et al.*, 1997; Eggert & Wallace, 2003). Additionally, detrital inputs vary seasonally as shown for a forested, headwater stream in New Hampshire with the bulk of inputs occurring in the fall and the majority of in-stream transport of detritus happening in the fall and spring months (Fisher & Likens, 1973). The aggregation of detritus in the winter and summer could therefore account for an increase in stream biomass by way of increasing detritivores. As noted above, terrestrial insects may constitute a significant fraction of stream fish consumption (see Baxter *et al.*, 2005; Utz & Hartman, 2007), but these subsidies will likely fluctuate through the year and so a logical hypothesis is that NBSS models will track these subsidies through time. Specifically, Baxter *et al.* (2005) observed that terrestrial insect inputs to streams display variable peaks depending on the region or even the year, although they concluded that

such inputs usually peak in the summer. As such, summer increases in fish biomass and individual size could lead to shallower slopes since fish are obtaining a significant proportion of their diet from terrestrial insects. Since year-to-year input fluctuations occur, the long-term experimental forests used in previous terrestrial studies (e.g., Hubbard Brook, New Hampshire and Coweeta Forest, North Carolina) may be the best places to study subsidy input timing and the impact on NBSS parameters. For instance, a future direction for NBSS in streams could focus on exclusion or removal of allochthonous inputs for an extended duration (e.g., Wallace *et al*. 1997) to determine whether the NBSS intercept, as an index of the total resource base within a stream, is significantly lower than in streams receiving allochthonous inputs and whether the slope changes in a predictable manner.

Spatial variability may explain NBSS parameter fluctuations as well. For example, substrate composition can drive body size distributions in aquatic systems (e.g. Lamouroux, Dolédec & Gayraud, 2004; Nash *et al.*, 2013). Bourassa and Morin (1995) examined NBSS variation relative to substrate composition in eastern Canadian streams. They noted that benthic invertebrate *D* remained relatively constant although the distributions were related to substrate composition and phosphorous concentrations. For instance, organisms weighing 0.001 mg were more prevalent in sandy substrates and less prevalent in large rocky substrates. However, the distribution of invertebrates >1 mg appeared to be consistent across substrate types (sand, fine gravel, coarse gravel, cobble, and rocks). To our knowledge, stream studies examining the effect of substrate composition on stream NBSS when fish are included do not exist.

Predictable changes in the NBSS may also be driven by upstream-downstream gradients in the structural and functional traits of local in-stream communities. One such framework for examining NBSS changes along a longitudinal gradient is the River Continuum Concept (RCC). The RCC describes biotic adjustments, namely community composition, in response to abiotic factors (i.e. width, depth, flow, temperature, and substrate) that change in a predictable manner as one moves from headwater streams to large rivers (Vannote *et al*., 1980). Additionally the RCC predicts that headwater streams and large rivers rely primarily on allochthonous inputs, whereas mid-order rivers may receive a substantial fraction of energy from aquatic autotrophs. Therefore, an enlightening line of investigation may be to gather localized $B \propto M$ data for a river basin from its headwater streams to its mouth to determine what, if any, NBSS patterns become apparent when observed over a longitudinal gradient. For instance, large rivers can support larger fishes than small streams and so it is reasonable to suspect that the intercept of the NBSS should demonstrably increase as stream order increases.

Viewing NBSS in context of the natural flow regime proposed by Poff *et al.* (1997) may offer some insight to how NBSS parameters could change. As noted above, a major flood occurred in March 2015 during our study at each of our streams. This was most apparent at Slaunch Fork and Cabin Creek where flooding dislodged and transported very large and entrenched tree trunks out of the stream reaches, no doubt scouring organisms within the streams as well. However, we did not observe a common NBSS pattern two months after the flooding. Exploring the shape of stream biomass spectra prior to and immediately following flooding has the potential to reveal interesting community dynamics. Because flooding washes organisms from their inhabited stream

segments, biomass spectra can be used to track the loss of biomass from the system. Additionally, biomass spectra may reveal the size distribution of organisms flushed from the system and reveal how long it takes for the biomass spectra to return to pre-flood levels. For instance, a flood over a prolonged duration may lead to the loss of riffle habitat, increasing the biomass of very small (~0.001 mg) invertebrates and possibly resulting in a steeper negative slope.

Finally, we note that the NBSS can potentially serve as a tool to characterize or predict disturbance effects. Rice and Gislason (1996) observed that the slope for size spectra in the North Sea became increasingly negative (-1.5) over a 20-year period as a result of overfishing. Jennings and Blanchard (2004) further demonstrated through theoretical NBSS models for the North Sea that fishing has likely resulted in a 38% reduction of biomass, relative to an unexploited system. More recently, Emmrich *et al.* (2011) showed that as nutrients and/or mean water depth increase, size spectra become steeper while size diversity decreases for lakes in Germany suggesting a greater *D* of larger predaceous fish. Therefore, it may be possible that nutrient enrichment causes predictable changes in the slope of the NBSS. The use and interpretation of size spectra alone may limit the efficacy of size spectra as an ecological indicator. And that has led Petchey and Belgrano (2010) to emphasize the importance of the inclusion of taxa specific life history in constructing size spectra as ecological indicators applicable across systems.

There is not yet an empirical baseline for stream NBSS, but the prevalence and severity of anthropogenic disturbance in the Southern Coal Fields could permit the exploration of what NBSS models look like in impaired streams. Bernhardt *et al.* (2012)

noted that as the extent of surface mining increased in the Southern Coal Fields region, so too did ionic concentrations associated with mining runoff (SO_4^{2-}, Ca^{2+}, Mg^{2+}, HCO_3^-), contributing to the loss of pollution-sensitive macroinvertebrate taxa. Pollution-sensitive taxa in small streams (e.g., mayflies, stoneflies, and caddisflies) often attain larger body sizes than more tolerant taxa (e.g., chironomids and blackflies). Thus, the distribution of biomass in an impaired stream may shift towards smaller, more tolerant taxa (see Johnson *et al.* 2013), ultimately leading to a steeper, more negative NBSS slope. Additionally, insights on how NBSS differ between unimpaired and impaired streams may benefit from the suggestion of Petchey and Belgrano (see above) to include a taxonomic element.

Further exploration of stream NBSS is needed to establish temporal and spatial trends comparable across lotic systems. The NBSS relationships in the three minimally impacted Appalachian streams in this study demonstrated some variability in the scaling of $B \propto M$ among sites and seasons. Specifically, size spectra intercept variability may reflect seasonal differences in fish and invertebrate densities. Yet, size spectra slopes were more consistent, suggesting a common scaling relationship between stream consumers at differing trophic levels. Small, forested lotic systems may be more naturally variable among sites and seasons than what has been shown for $B \propto M$ scaling in lake and marine systems. Moreover despite inter-stream variability, NBSS models are comparable across systems and can be related to previously established lotic-specific phenomena.

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Tables and Figures

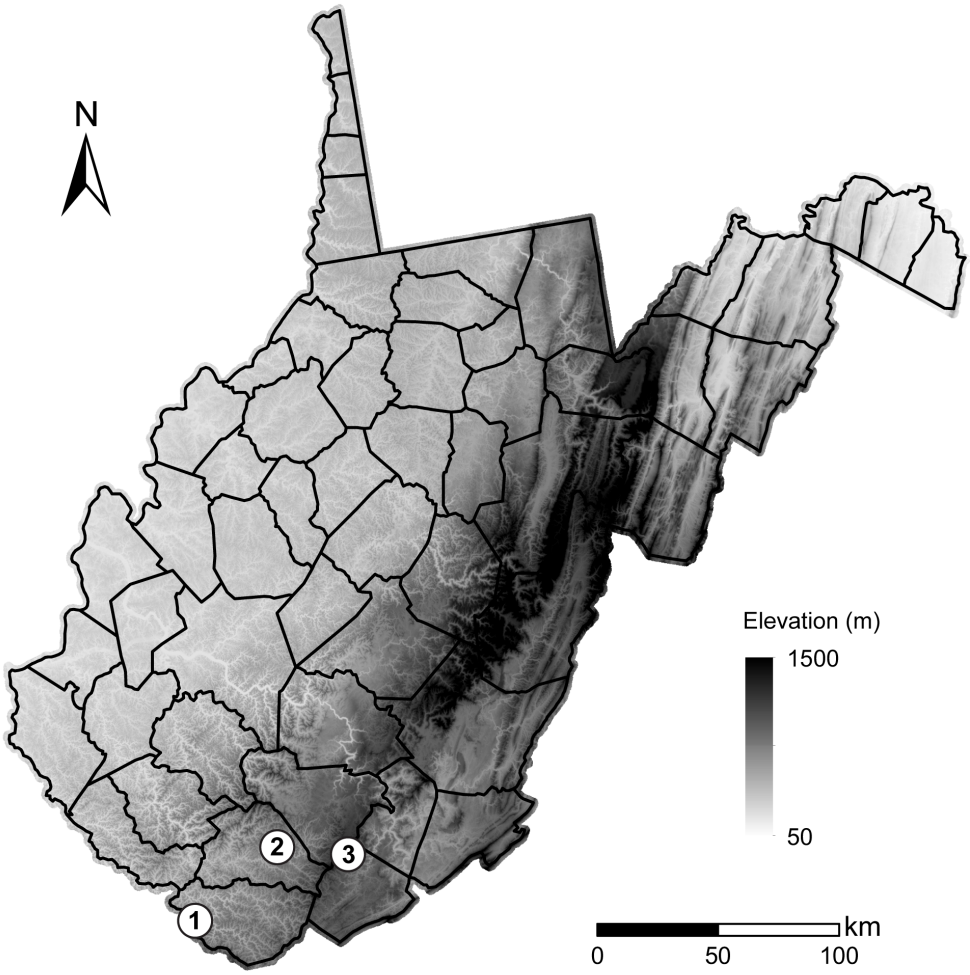


Figure 1. Topographic map of West Virginia with county boundaries (black lines). Slaunch Fork (1), Cabin Creek (2), and Camp Creek (3) locations are denoted by white, numbered circles.

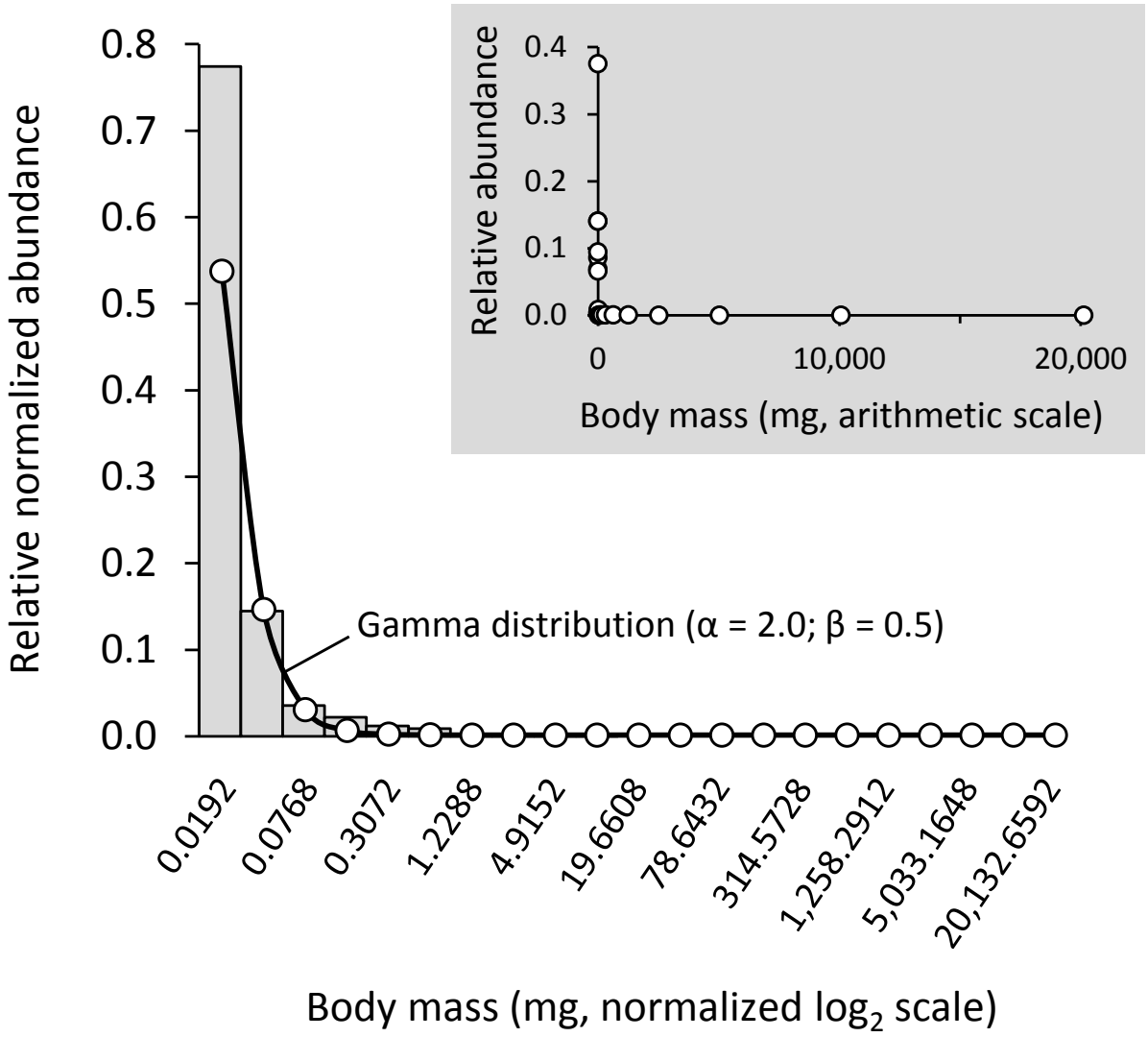


Figure 2. Histogram showing the distribution of individual macroinvertebrates and fishes within $\log_2$ *M* bins. The main panel depicts normalized abundance and *M* data without $\log_{10}$ transformation; the $\log_2$ *M* bins are plotted as equal-interval units (*x* axis, with integer scale values shown in parentheses) against the normalized abundances (*y* axis), revealing the exponential shape of the abundance versus *M* relationship. In this instance, the relationship is well-approximated by a gamma distribution with α and β parameters of 2.0 and 0.5, respectively. The inset panel shows the same data when normalization has not been applied. The geometric increase in *M* values along the *x* axis completely obscures the underlying distribution of abundance and *M*. Data are shown for the October 2013 macroinvertebrate and fish samples from Slaunch Fork, West Virginia.

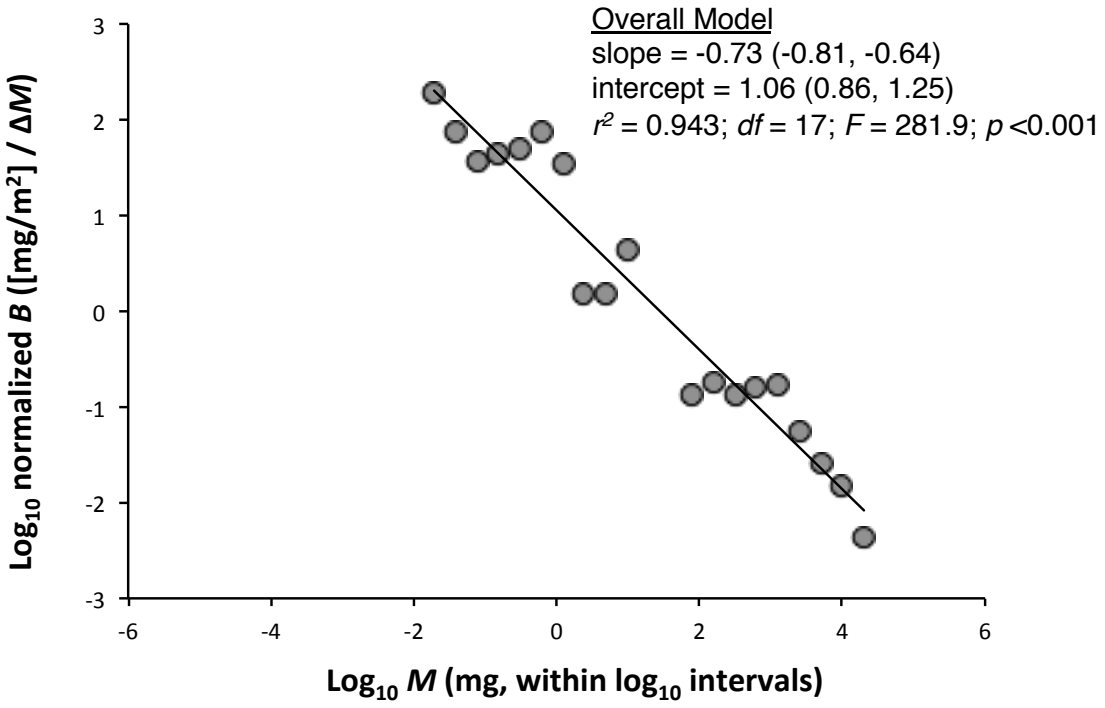


Figure 3. Normalized biomass size spectrum (NBSS) model for fish and benthic macroinvertebrate data from Slaunch Fork, West Virginia (October 2013 sample). Ordinary least squares regression was used to model the relationship between average body mass within $\log_2$ size intervals (*M*, mg dry weight) and total normalized biomass (*B*) as log (*B*) = log (*a*) + *b* log (*M*). The least squares linear regression line is shown for the overall NBSS. The linear model slope, intercept, coefficient of determination (r^2), degrees of freedom (*df*), *F*-statistic (*F*), and significance level (*p*) are shown in upper right corner of the figure, with 95% confidence bounds shown in parentheses for the slope and intercept terms.

Table 1. Summary statistics for all Normalized Biomass Size Spectra models. Overall linear model results (log_{10} transformed data) are shown for each Site × Date combination, including *F*-statistics (*F*), coefficients of determination (r^2), and significance levels (*P*). Individual results are also shown for the linear model slopes and intercepts, including the parameter estimates (Est.), standard errors (Std. Err.), and significance levels (*p*) for each model term. Significance levels of '<0.01' indicate very small values (≤0.0001).

		Overall model			Slope (*b*)			Intercept (*a*)		
Site	**Date**	***F***	***r²***	***P***	**Est.**	**Std. Err.**	***p***	**Est.**	**Std. Err.**	***p***
Cabin Creek	October 2013	132.95	0.89	<0.01	-0.74	0.06	<0.01	0.80	0.15	<0.01
	March 2014	114.13	0.90	<0.01	-0.70	0.07	<0.01	0.01	0.15	0.93
	May 2014	164.07	0.90	<0.01	-0.88	0.07	<0.01	0.82	0.17	<0.01
	August 2014	27.62	0.68	<0.01	-0.53	0.10	<0.01	0.03	0.26	0.91
	October 2014	88.50	0.86	<0.01	-0.59	0.06	<0.01	0.32	0.15	0.06
	May 2015	279.49	0.96	<0.01	-0.64	0.04	<0.01	0.42	0.10	<0.01
Camp Creek	October 2013	296.28	0.94	<0.01	-0.76	0.04	<0.01	0.80	0.11	<0.01
	March 2014	164.23	0.90	<0.01	-0.81	0.06	<0.01	0.72	0.17	<0.01
	May 2014	159.58	0.90	<0.01	-0.85	0.07	<0.01	0.96	0.17	<0.01
	August 2014	154.23	0.90	<0.01	-0.78	0.06	<0.01	0.78	0.14	<0.01
	October 2014	144.03	0.91	<0.01	-0.77	0.06	<0.01	0.34	0.14	0.03
	May 2015	122.67	0.89	<0.01	-0.80	0.07	<0.01	0.87	0.14	<0.01
Slaunch Fork	October 2013	281.87	0.94	<0.01	-0.73	0.04	<0.01	1.05	0.10	<0.01
	March 2014	359.65	0.96	<0.01	-0.78	0.04	<0.01	1.04	0.10	<0.01
	May 2014	172.38	0.91	<0.01	-0.80	0.06	<0.01	1.20	0.16	<0.01
	August 2014	185.72	0.91	<0.01	-0.62	0.05	<0.01	0.63	0.10	<0.01
	October 2014	189.80	0.92	<0.01	-0.73	0.05	<0.01	0.80	0.13	<0.01
	May 2015	156.31	0.91	<0.01	-0.62	0.05	<0.01	0.62	0.12	<0.01

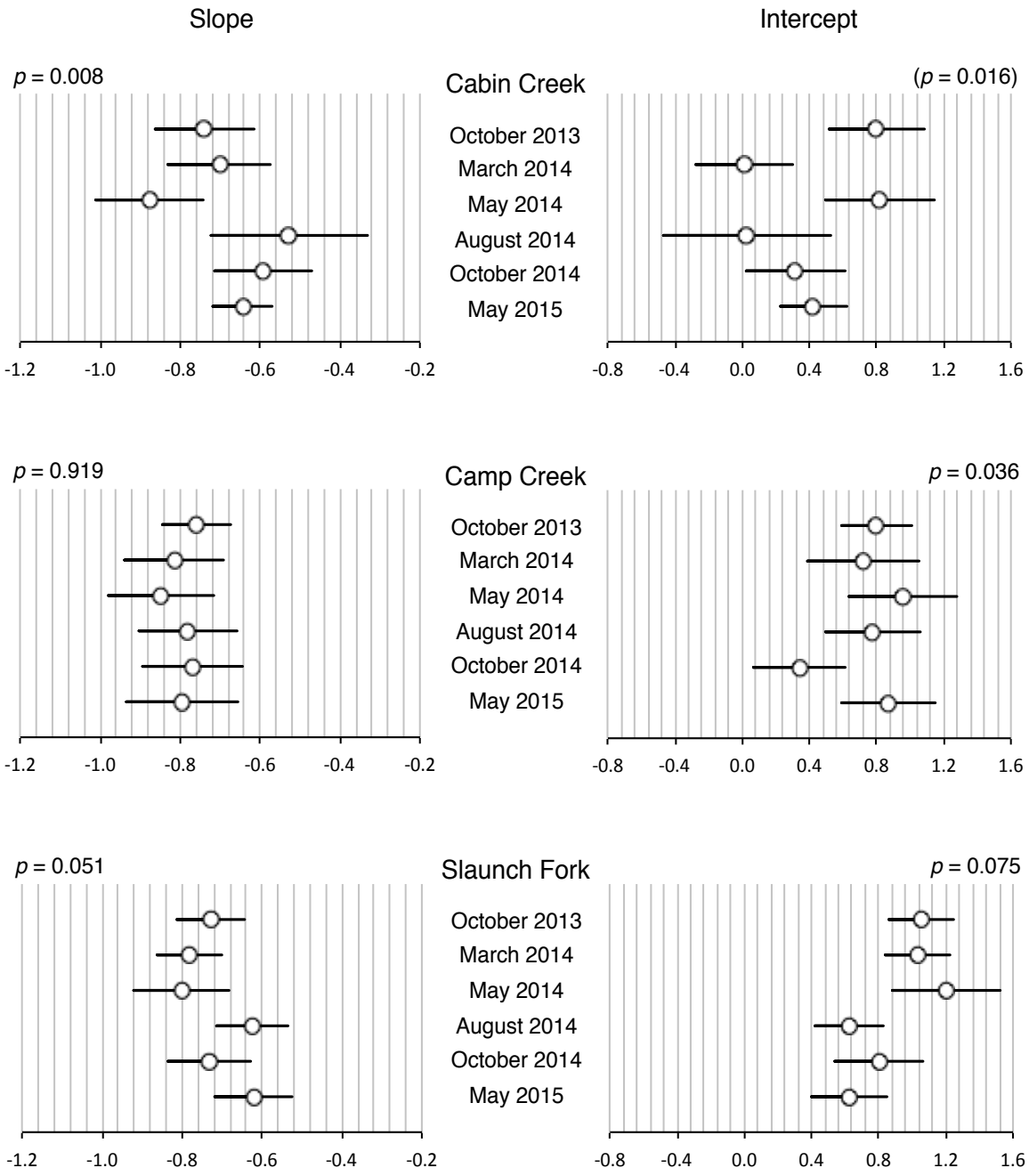


Figure 4. Slopes and intercepts of normalized biomass size spectra (NBSS) models, estimated for each stream × season sampling event. Estimated parameters are represented by white circles with 95% confidence intervals represented by black lines. ANCOVA *p*-values for the combined set of seasonal NBSS models from each of the three study streams are shown in the upper right or left corner of each plot. The ANCOVA *p*-value for NBSS model intercepts in Cabin Creek is shown in parentheses to emphasize that a formal test of differing intercepts is not valid when the slopes were previously found to be significantly different. Grey vertical tics were added for ease of comparison.

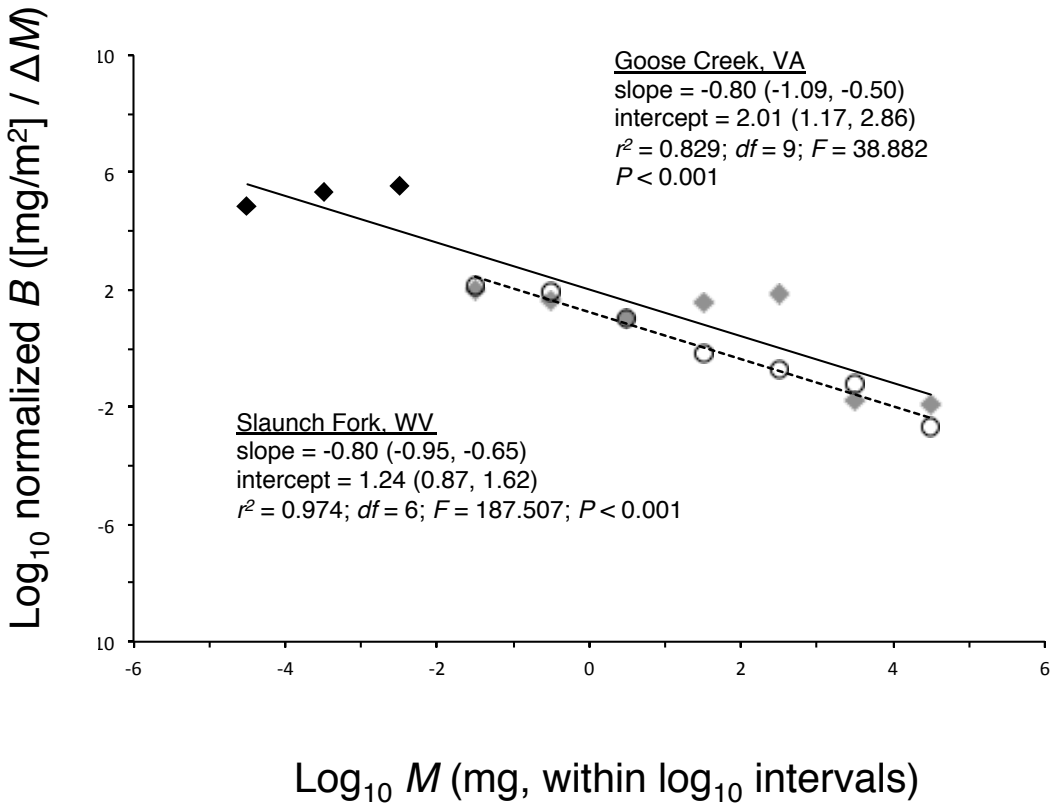


Figure 5. Comparison of body size scaling relationships in Slaunch Fork and Goose Creek, Virginia (see Poff et al. 1993). Regression model results (log-log transformed data) are included for normalized biomass (*B*) facilitate direct comparisons, the raw invertebrate and fish data from Slaunch Fork were first re-assigned to $\log_{10}$ *M* (mg dry mass) intervals. This was necessary because Poff et al. (1993) used $\log_{10}$ *M* intervals and we did not have access to their raw data. Thus, we were not able to re-assign the Poff et al. (1993) data to $\log_2$ *M* intervals. The *B* estimates for Goose Creek and Slaunch Fork were then normalized with ΔM set to $\log_{10}$, rather than $\log_2$, intervals. Slaunch Fork data are shown as open circles. Goose Creek data are shown as shaded diamonds: grey diamonds indicate macroinvertebrate and fish data that occupy the same *M* intervals as the Slaunch Fork samples, while black diamonds indicate meiofauna samples that were collected with cores in Goose Creek, but not in Slaunch Fork.

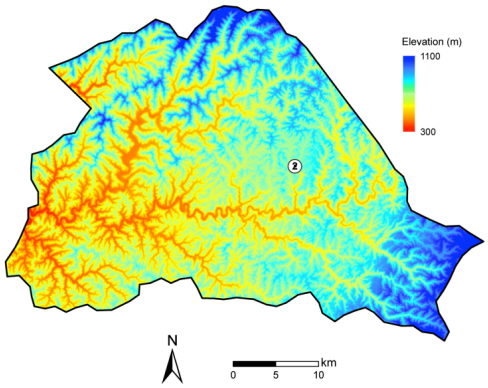
Appendix S1

Study Site Summaries

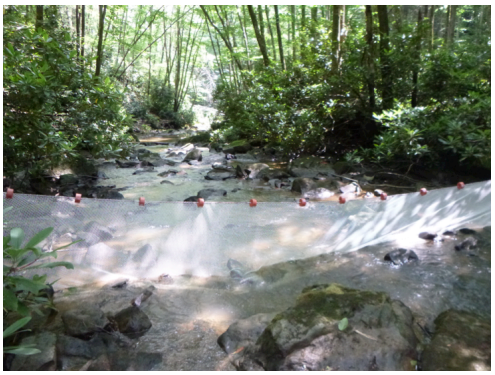
Cabin Creek
Wyoming County, West Virginia

Fish species: 11 Macroinvertebrate taxa: 38

Latitude	37.617
Longitude	-81.454
Basin (km^2)	18.46
Mean wetted width (m)	10.7
Survey Reach (m)	100
Surface area (m^2)	1097
Mean gradient (%)	5.2
Appalachian Stream Classification	Perennial Runoff, Transitional Cool, Medium Gradient, Stream



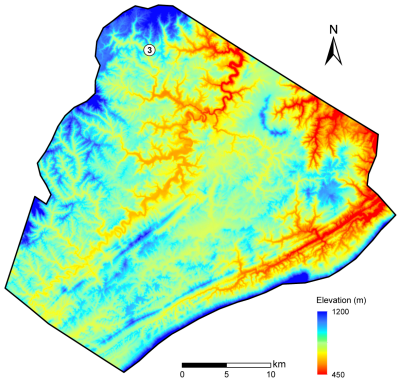
Environmental Variable	October 2013	March 2014	May 2014	August 2014	October 2014	May 2015
Water temperature (°C)	14.1	4.4	14.4	17.5	11.3	16.4
pH	5.5	5.0	6.0	6.4	5.0	5.0
Specific Conductivity(µg/cm)	117.3	77.9	83.1	116.2	99.9	167.5
Dissolved Oxygen (mg/L)	6.72	11.83	8.95	8.60	9.65	8.33



Camp Creek
Mercer County, West Virginia

Fish species: 10 Macroinvertebrate taxa: 54

Latitude	37.55
Longitude	-81.131
Basin (km^2)	26.05
Mean wetted width (m)	13.4
Survey Reach (m)	153
Surface area (m^2)	2012
Mean gradient (%)	3.2
Appalachian Stream Classification	Perennial Runoff, Transitional Cool, Medium Gradient, Stream

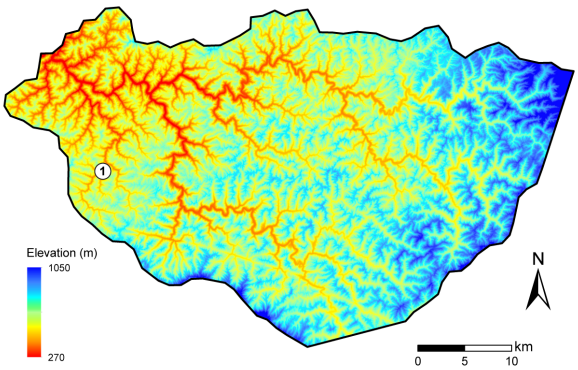


Environmental Variable	October 2013	March 2014	May 2014	August 2014	October 2014	May 2015
Water temperature (°C)	13.2	5.4	15.6	16.5	12.4	16.4
pH	6.4	4.5	5.0	5.5	5.0	5.0
Specific Conductivity(µg/cm)	169.5	141.4	92.6	206.2	71.3	130.3
Dissolved Oxygen (mg/L)	7.64	11.60	9.05	7.20	9.37	8.30

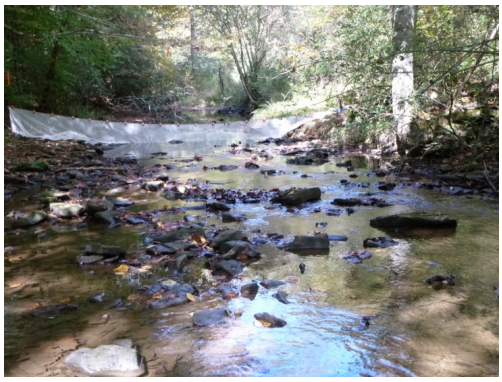
Slaunch Fork
McDowell County, West Virginia

Fish species: 19 Macroinvertebrate taxa: 60

Latitude	37.396
Longitude	-81.889
Basin (km^2)	35.4
Mean wetted width (m)	11.3
Survey Reach (m)	185
Surface area (m^2)	2093
Mean gradient (%)	2.3
Appalachian Stream Classification	Perennial Flashy, Transitional Cool, Medium Gradient, Stream



Environmental Variable	October 2013	March 2014	May 2014	August 2014	October 2014	May 2015
Water temperature (°C)	11.6	5.3	15.4	18.6	12.8	18.7
pH	6.4	5.5	6.4	6.4	---	6.4
Specific Conductivity(µg/cm)	304.6	77.6	203.1	171.5	131.7	393.5
Dissolved Oxygen (mg/L)	8.61	11.40	8.62	7.66	9.30	8.00



Appendix S2.

Complete description of the depletion procedure that was used to estimate fish abundances for taxic and ataxic data for Slaunch Fork, October 2013.

Taxic (species-level) abundance estimates

Abundance (n) of each fish species was estimated using species' counts from the 3-pass survey and the Zippin (1958) depletion method. Specifically, we used the maximum-likelihood procedure of Carle and Strub (1978) to first calculate an intermediate statistic X as:

$$X = \sum_{i=1}^{k}(k-i)C_i\,, \qquad \text{(S2.1)}$$

where i is the i^{th} sampling pass (i = 1, 2, or 3), k is the total number of passes (k = 3), and C_i is the total number of fish caught (of a given species) in the i^{th} pass. The maximum-likelihood estimate of n was then calculated iteratively by substituting decreasing n values until:

$$\left[\frac{n+1}{n-T+1}\right]\prod_{i=1}^{k}\left[\frac{kn-X-T+1+(k-i)}{kn-X+2+(k-i)}\right]_i \leq 1.0, \qquad \text{(S2.2)}$$

where T is the total number of individuals (of a given species) caught in k passes and all other variables are as defined above for Eq. S2.1. The n estimates were divided by the wetted surface area of the surveyed stream channel (2,093 m^2) to obtain species' density (D) estimates at the per-square meter scale for direct comparison with the benthic macroinvertebrate data. Total observed abundances (summed counts among the three passes) were used as our n estimates when zero counts were observed in the first, second, or third pass for a given species.

For example, we captured 128 (C_1), 94 (C_2), and 54 (C_3) blacknose dace (*Rhinichthys atratulus*) in electrofishing passes 1, 2, and 3, respectively. Following Eq. A.1, X = 350 when k = 3 passes. Eq. A.2 was then solved by iteration, resulting in n = 380 when T = 128 + 94 + 54 = 276. Blacknose dace D was then estimated by dividing n by the stream channel surface area (D = 380 ÷ 2,093 m^2 = 0.182 individuals/m^2). Counts of each species collected in electrofishing passes 1, 2, and 3 are listed in Table S2.1 with the resulting Zippin n estimates.

Ataxic (pooled individuals within $\log_2$ dry mass bins) abundance estimates

We also estimated *n* for fishes within nine $\log_2$ body mass (*M*) bins (52.43–104.85; 104.86–209.71; 209.72–419.42; 419.43–838.85; 838.86–1,677.71; 1,677.72–3,355.43; 3,355.44–6,710.88; 6,710.89–13,421.76; 13,421.77–26,843.54 mg dry mass). This procedure was logically consistent with the ataxic intent of Kerr and Dickie (2001); it treated individual fishes as 'particles of given size' without including any information on species' identifications.

To calculate ataxic *n* estimates, we used Eq. S2.1 and Eq. S2.2 as above, substituting counts of individuals within the $\log_2$ *M* bins for counts of species. For instance, we captured 61, 37, and 31 individuals ($T = 129$) within the 52.43–104.85 $\log_2$ *M* dry mass interval. Following Eq. S2.1, *X* for this series was 159. Eq. S2.2 was then solved as above, resulting in $n = 185$ and $D = 0.088$ individuals/m^2. Counts of individuals collected within each $\log_2$ *M* bin, distributed among the three electrofishing passes, are listed in Table S2.2.

Table S2.1. Counts of individual fishes collected in electrofishing passes 1, 2, and 3 summarized by species (i.e., taxic data).

Species	**Pass 1**	**Pass 2**	**Pass 3**	***n***
Northern hog sucker (*Hypentelium nigricans*)	6	5	3	23
White sucker (*Catostomus commersonii*)	28	4	1	33
Mottled sculpin (*Cottus bairdii*)	64	65	20	3379
Blacknose dace (*Rhinichthys atratulus*)	128	94	54	380
Central stoneroller (*Campostoma anomalum*)	9	6	6	41
Creek chub (*Semotilus atromaculatus*)	65	61	60	2202
River chub (*Nocomis micropogon*)	6	0	0	6
Rosyside dace (*Clinostomus funduloides*)	54	26	11	101
Banded darter (*Etheostoma zonale*)	14	3	3	20
Fantail darter (*Etheostoma flabellare*)	5	1	2	8
Johnny darter (*Etheostoma nigrum*)	0	10	2	12

Table S2.2. Counts of individual fishes collected in electrofishing passes 1, 2, and 3 organized by $\log_2$ *M* bins (i.e., ataxic data). Log_2 *M* bins are identified by their respective *M* midpoints (e.g., '78.64' represents the 52.43–104.85 *M* interval).

Log_2 *M* bin	Pass 1	Pass 2	Pass 3	*n*
78.64	61	36	31	183
157.29	26	40	25	262
314.57	63	49	24	178
629.15	88	52	34	222
1,258.29	78	66	29	229
2,516.58	29	17	15	84
5,033.16	18	11	3	34
10,066.33	16	4	1	21
20,132.66	5	1	0	6

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Carle, F. L. and M. R. Strub. 1978. New method for estimating population-size from removal data. Biometrics 34:621-630.

Kerr, S. R. and L. M. Dickie. 2001. The biomass spectrum: a predator-prey theory of aquatic production. Columbia University Press, New York.

Zippin, C. 1958. The removal method of population estimation. The Journal of Wildlife Management 22:82–90.